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Biohazardous Materials Safety Manual

BIOHAZARDOUS MATERIALS SAFETY MANUAL

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INTRODUCTION

School boards and their agents, classroom teachers, have an obligation to ensure their classrooms are free from hazardous materials that might endanger students, other workers and visitors. In laboratory exercises and demonstrations involving cells, tissues or organisms, it is impossible to guarantee an absolutely sterile environment for all students and teachers at all times. It is, then, wise to prohibit the use of potentially dangerous biological materials during laboratory work and demonstrations done in the classroom. This is particularly important because some infectious organisms have been found to survive long periods of drying.

Concerns have been raised by the Canadian medical community regarding the handling of mammalian tissues, partially in response to the emergence of pathogens such as the virus which causes Acquired Immune Deficiency Syndrome (AIDS). AIDS and other diseases such as hepatitis which are transmitted through direct cellular contact by the intrusion of foreign fluids have been diagnosed in large numbers throughout the world. Guidelines and procedures are set out in this guide to help ensure the health and safety of students and staff who are studying the biological sciences.

The education value of investigations such as blood sampling or cheek cell scrapes can not justify the increased health risk of these and other related procedures to students and staff. The Nova Scotia Department of Education firmly believes that the health and well-being of all members of the education community must be a prime consideration in selecting learning activities.

Listed below is a summary of the major guidelines. Please ensure, however, that the entire document is read for more detail. For further clarification or information, please contact the Science Consultant, Nova Scotia Department of Education.

GUIDELINES

1. Students and teachers are to be made aware of procedures for surface decontamination and biohazardous waste disposal.

2. Students will be taught that handling body substances of unknown status could endanger their health.

3. The importance of scrubbing the hands at the end of every laboratory session should be stressed. School boards are to ensure that adequate facilities are available for students and staff to wash their hands.

4. Staff and students will follow the Canadian Council For Animal Welfare general rules and regulations when they have animals in their classrooms and laboratories.

5. The use of mammalian blood in school science classes is **prohibited**.

6. The use of urine and fecal material in classroom laboratory procedures is **prohibited**.

7. Laboratory procedures involving cheek cell scrapings or similar human cell or tissue sampling are **prohibited** in all school science classes.

8. The use of mammalian tissue, either fresh or frozen, is **prohibited** in all school science classes.

9. The use of human saliva in school science classes is **limited** to the careful use of sterile technique with swabs and Petri dishes.

10 Schools will safely **dispose** of their present stocks of formaldehyde.

GENERAL CLARIFICATION OF STATEMENTS

Current medical research clearly shows that the Canadian population has a number of asymptomatic hepatitis B virus carriers as well as individuals infected with the AIDS virus HIV-1 and other serious contagions, and that these numbers are on the increase. It is therefore essential that the educational system inculcate the concept of proper hygiene in the handling of human body substances in accordance with the current understanding of transmission of these infectious agents. **Students must be taught that handling body substances of unknown status could endanger their health.**

Although the risk of handling fecal material and urine has been stressed in the past, it is now evident that blood poses an even greater hazard. For this reason it is no longer permissible to expose students to accidental contamination by any human body substance. Because of the significant risk involved, **any experimentation with, or handling of, any human tissue is now strictly prohibited.**

In a survey of the laboratory exercises found in basic texts and laboratory manuals for Nova Scotia science courses, contentious exercises identified fall into four major groups: those involving blood, urine, saliva and tissue sampling. General and specific modifications and substitutions are suggested for the four major groups. Because teachers often use a variety of teaching materials, it is not possible to list all possible laboratory procedures and exercises. It is the **responsibility of the teacher and administrative staff to ensure that all procedures are safe.**

Standard laboratory safety practices relating to these areas, and specific procedures for recommended modifications and substitutions, are included in Appendix I and Appendix II . From these guidelines, teachers can apply the recommendations to the laboratory manuals and texts they use. These guidelines apply to all curricular materials used in Nova Scotia schools even if those manuals and textbooks are not referred to directly in this document or on the List of Authorized Instructional Materials.

SPECIFIC STATEMENTS OF RISK

BLOOD AND URINE

Laboratory Exercises Involving the Chemistry of Urine and the Analysis of Blood

Because of the risk of contracting hepatitis, AIDS and other transmitted diseases, **the use of urine, blood and fecal material in classroom laboratory exercises is now prohibited.** Hepatitis B virus (HBV) and Human Immunodeficiency Virus Type 1 (HIV-1) or Type 2 (HIV-2) are known to be transmitted via contaminated blood contact with open lesions or mucous membranes. Semen, which can be found in urine, is known to have the highest concentration of AIDS virus of all human body substances. These facts make these materials for laboratory exercises too risky for their educational value and the risk is increasing as the number of HIV-1 or HIV-2 cases increases.

Substitution Suggestions

1. It is strongly suggested that teachers substitute blood from fish, amphibians or reptiles. The procedure is included in Appendix IIb. If this is not possible, then use prepared human blood smear slides for observation of cellular composition. The principles of good biology teaching strongly suggest using substitute blood rather than simply viewing prepared slides.

2. Use videos, films and slides that show typical laboratory procedures involved in human urinalysis, blood typing and blood analysis.

3. Use samples of synthetic urine for laboratory exercises involving testing for the presence of glucose or salts. The procedure is included in Appendix IIc.

SALIVA

Salivary Amylase Experiments

Human saliva may contain hepatitis B virus or the tuberculosis bacillus. Saliva is not known to transmit the etiologic agent for AIDS although HIV-1 has been found in saliva. The collection and manipulation of saliva is extremely difficult to execute in a sanitary manner in the classroom setting. It is one matter to have small amounts of saliva soaked into a swab and quite another to collect saliva in test tubes and then manipulate it. **The use of human saliva for salivary amylase or other experiments involving more than minute quantities of saliva on a swab and placed in a Petri dish is now prohibited.**

Synthetic enzyme is available and is a safe substitute for salivary amylase in laboratory exercises examining the action of this enzyme.

Swabs Used in the Mouth

All used swabs should be placed on disposable absorbent pads and both pads and swabs disposed of, as biohazardous material, by incineration or autoclaving. Surfaces touched by swabs should be decontaminated. Hands should be washed thoroughly both before and after the exercise. Students with open wounds in their oral cavity, or bleeding gums, should not participate in exercises involving swabbing of the mouth area.

Care must be taken to ensure that fresh swabs are used for each sampling or test taken. (See surface decontamination and biohazardous waste disposal procedures in Appendix I.)

Testing the Effectiveness of Mouthwash

Students must not be allowed to take each other's samples. The decontamination of desk tops or laboratory bench tops after the exercise is important. The use of absorbent pads is highly recommended for this lab. Disposal of the swabs and these absorbent pads should be by incineration. The students must be instructed to take special precautions to measure the zones of inhibition by the mouthwashes from the bottom of the Petri dish, not by opening the lid. The disposal of the culture dishes should be either by incineration for disposable plastic Petri dishes or by autoclaving for glass Petri dishes. All wastes or contaminated materials must be well labelled with biohazardous materials labels or enclosed in biohazardous logo garbage bags (orange in color).

The importance of scrubbing the hands at the end of every laboratory session should be stressed. School boards are to ensure that adequate facilities are available for students and staff to wash their hands. (See surface decontamination and biohazardous waste disposal procedures in Appendix I.)

PTC Testing

The chemical phenylthiocarbamide (PTC), although considered a toxic substance, is toxic only at a much higher dosage than one would receive from a simple lick of PTC paper. The LD_{50} ranges from 3 to 40 mg/kg. However, students must be warned and closely supervised to ensure no person tastes a piece of paper previously tasted, and that the papers are disposed of properly. Do not allow students to set used papers down on lab benches or desks; otherwise the surfaces will have to be decontaminated.

Lung Capacity Investigation/Gas Exchange

When a spirometer is used, a fresh sterile mouthpiece is required for **each** student. Mouthpieces, if disposable, are to be placed in a biohazard label bag by the student for incineration or autoclaving. If the mouthpiece is to be decontaminated by

soaking in 10% bleach solution or Presept solution, it should be deposited by the student directly into the solution.

Straws/Paper Bags/Balloons

When balloons or straws or paper bags are used, proper instruction and supervision are necessary to avoid these materials being shared or saliva becoming airborne. Proper disposal of paper bags or balloons or straws is essential, and surface decontamination procedures should be followed if these items are placed on laboratory surfaces. (See surface decontamination and biohazardous waste disposal procedures in Appendix I.)

HUMAN TISSUE

The most common laboratory exercise for observation of typical human cells and their staining properties involves cheek cell scrapings. **This practice is no longer acceptable.** As a substitute, to illustrate simple staining technique and general animal cell characteristics, a variety of tissues from fish, amphibians or reptiles should be used. The procedure for preparation of fresh tissue samples from fish, amphibians or reptiles is given in Appendix II along with slide preparation and staining techniques. Permanently fixed slides of human tissues can be used to show human cell characteristics through demonstration, or as a supplementary laboratory procedure.

SHEEP TISSUE

The use of sheep tissues could be considered especially biohazardous. There is abundant literature to indicate that the causative organism for Q-fever is widely distributed in sheep flocks in North America and in most parts of the world. This is most important for individuals who have heart valve problems, those who are immuno-suppressed, and perhaps those who are pregnant. Consequences of the illness range from fever and symptoms similar to flu, to fatality. Although sheep red blood cells have been found to contain the organism for Q-fever, blood from sheep has never been reported as causing the infection. **Nevertheless, sheep tissue is not to be used under any circumstances.**

USE OF OTHER MAMMALIAN TISSUE (FRESH OR FROZEN)

Because it is impossible for the teacher to ascertain if any student or staff member has a heart valve problem or is immuno-suppressed, **mammalian tissue either fresh or frozen is banned from all science laboratories and classrooms.** The concern is that infectious agents can become airborne.

Scientific supply companies can provide teachers with a wide variety of preserved mammalian tissues as a substitute for fresh or frozen mammalian tissues. It is strongly suggested that teachers and boards purchase physically preserved (sublimation process) specimens because most chemically preserved specimens use formaldehyde. Recent evidence has suggested a relationship between formaldehyde and cancer of the nose. In addition, some students may exhibit various allergic

reactions to the irritation of the nose, throat, eyes and skin by formaldehyde fumes. If chemically preserved specimens are used, it should be ensured that the preserving fluid has no formaldehyde. Several companies use alcohol solutions and these are acceptable for use.

Formaldehyde has been a restricted chemical in Nova Scotia schools. It is now **prohibited.** All schools are to properly dispose of their present stocks and use substitutes such as alcohol solutions.

APPENDIX I (a)

(a) Surface Contamination Procedures

(b) Biohazardous Materials Identification

(c) Disposal of Biohazardous Materials

(a) SURFACE DECONTAMINATION PROCEDURES

Standard Procedure

Wipe area down well with a freshly made 10% bleach solution (approximately 10,000 ppm or 10,000 mg/L of available chlorine).

(b) BIOHAZARDOUS MATERIALS IDENTIFICATION

Labelling and Proper Disposal Technique

All biohazardous garbage to be incinerated or autoclaved must be clearly identified by placing in special orange biohazard logo garbage bags or by attaching a clearly visible biohazard label. For materials to be autoclaved, temperature sensitive autoclave tape bags or temperature sensitive biohazardous logos should be used. The autoclave tape or logo changes color when autoclaved, clearly indicating the contents have been sterilized. Sharp instruments such as lancets, syringes, scalpel blades or razor blades must be placed in appropriate biohazard canisters. **When full, these canisters should be incinerated or autoclaved and then disposed of in a landfill site.**

Biohazardous Logo

Biohazardous Labels

Biohazardous cannisters for sharp instrument disposal, garbage bags with biohazardous logo, autoclave tape (sterilizer indicator tape), and biohazardous labels are readily available from scientific supply houses.

Culture Plate Procedures

Use of agar plates is a simple method of culturing bacteria. When classroom procedures involve random sampling of unknown materials, caution must be taken to avoid direct contact with cultured colonies in order to avoid accidental exposure to pathogenic organisms.

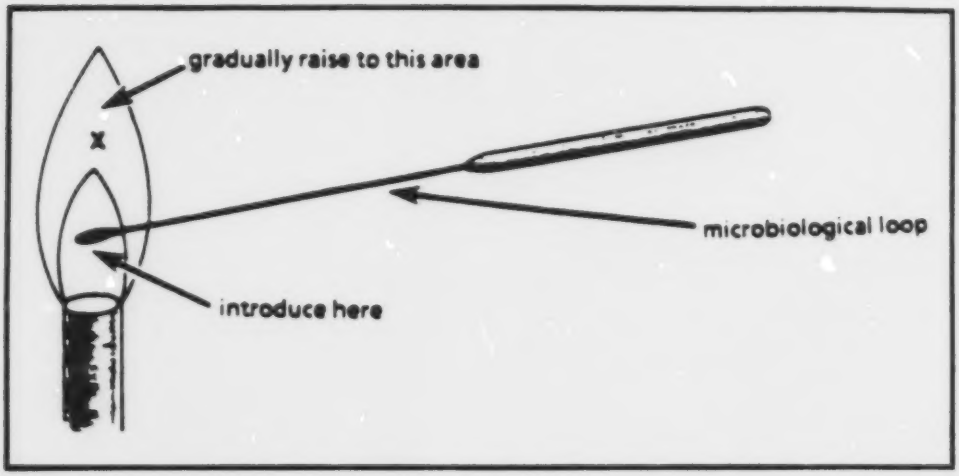
Immediately after streaking, swabs used for sampling should be disposed of in a biohazard garbage bag, for incineration or autoclave sterilization. Bench top surfaces should be decontaminated at the end of each laboratory session. (See surface decontamination procedures in Appendix (a).) Staff and students should develop the **habit** of washing their hands thoroughly with soap and water at the end of the exercise.

As soon as the agar surfaces of the culture plate have been streaked, the lid should be taped to the bottom half of the Petri plate to prevent accidental contact with cultured organisms.

Clearly labelled Petri plates should be incubated upside down, in stacks which are then secured in a plastic bag.

All observations of cultured colonies are to be done without opening the taped plates. Colonies are clearly visible through the lid, and zones of inhibition can be measured from the bottom.

If samples of cultured colonies are to be taken for fixing, staining and microscopic observation, the proper sampling techniques using a microbiological loop must be followed. Lid lifting is minimized, with the loop being flamed before sample-taking and after placement of the sample on the slide. Proper flaming of the loop involves introducing the loop into the cooler middle cone of the Bunsen burner flame and then slowly raising it into the hotter, exterior region of the flame. (See diagram below). This prevents the spattering of possible pathogens before they are killed by the heat.



(c) DISPOSAL OF BIOLOGICAL MATERIALS

All microbiological specimens and dissected organisms should be incinerated. If this procedure is not possible, the remains of animals are to be carefully sealed in heavy biohazard bags and sent to a landfill site. Microrganism cultures should be autoclaved, sealed in a biohazard bag and sent to a landfill site. Liquid cultures should be autoclaved and flushed down the drain with large amounts of water. School Boards should identify autoclaves and incinerators which can take these material.

Transportation of infectious materials is regulated by the Transportation of Dangerous Goods Act and Regulation. Inquiries regarding transportation of infectious materials should be directed to the Department of Transportation and Communications.

APPENDIX II

a) Canadian Council for Animal Welfare - General Rules and Regulations.

b) Procedures for Obtaining Fresh Tissue Samples from Fish, Amphibians and Reptiles.

c) Procedure for Making Synthetic Urine

APPENDIX II (a)

CANADIAN COUNCIL FOR ANIMAL WELFARE - GENERAL RULES AND REGULATIONS

The following material is based on excerpts from:

Canadian Council on Animal Care
Guide to the Care and Use of
Experimental Animals.
2 vols
Ottawa, Ont.: CCAC, 1980-1984.

Volume I - outlines the ethics and philosophies of animal care including health and safety responsibilities, laboratory animal facilities, euthanasia and responsibilities for the care of experimental animals

Volume II - outlines the care, housing and management of individuals as well as suitable experiments at the pre-university level. It is recommended that every school having animals should have and use this resource to guide procedures.

Copies may be obtained from:

Canadian Council on Animal Care
1000-151 Slater Street
Ottawa, Ontario
K1P 5H3
Telephone: (613) 238-4031

Approximate cost of the two volumes is $9.00 including postage.

Preface

The Canadian Council on Animal Care (CCAC) is an independent, incorporated body, supported financially by the Natural Sciences and Engineering Research Council (NSERC) and the Medical Research Council (MRC).

An Executive Director and a Director of Assessments head the CCAC secretariat which, with the assistance of its committees, serves as a resource and information centre for those using experimental animals, as well as for the general public. Assistance is also offered to persons responsible for the care and use of animals in pre-university educational programs.

The fundamental concept on which animal care in Canada and its surveillance by CCAC is based, is that of control from within an institution, exercised by the scientists themselves in association with non-animal users from other faculty departments, and representatives of the community. This implies the creation of local animal care committees with specific authority, terms of reference and responsibilities, for assuring that all matters concerning the use and day-to-day care of experimental animals within institutions are carried out in accordance with the recommendations of CCAC.

A 110-member pool of scientists from universities, government, and the pharmaceutical industry, aided by representatives appointed by the Canadian Federation of Humane Societies (CFHS), is responsible for the implementation of the CCAC nation-wide assessment program. This system of periodic surveillance provides support to institutional animal care committees, uniformity of national standards and a means of keeping the agencies represented on the CCAC properly informed. Assessment panel and committee members are chosen for their expertise in a variety of fields, and their leadership and concern in matters of animal care and use. They serve without remuneration.

Both the French and English editions of [the CCAC] guide have become internationally known. These have provided a basis for assessment evaluations across Canada. Moreover, adherence to the principles enunciated in the guide, and a satisfactory assessment, are now required by all major national granting agencies. This is also a requisite for the awarding of contracts involving research animals by the Federal Departments of Health and Welfare, Agriculture, Environment and National Defence.

Legislation

CCAC's system of self-regulation by and with the full support of the scientific community, government agencies and the Canadian Federation of Humane Societies, is designed as an alternative to the necessity for national legislation of the care of experimental animals.

Federal legislation controlling the conditions under which animals must be transported is included in the "Animal Disease and Protection Act".

The Scientist-Teacher

The scientist-teacher should have knowledge of the characteristics, care and handling of the species he or she proposes to use and be committed to comply with the guidelines for care and ethical use of animals as contained in this guide.

The ultimate responsibility for the prevention of pain and discomfort in the experimental situation lies with the investigator.

Pre-University

There is sometimes a need for animals in the classroom at the pre-university level. This makes it possible to teach principles of animal care to students, so that they may better understand the normal functional and behavioral aspects of animals, as well as to inculcate a proper sense of responsibility toward animals and their care.

Unlike the investigator or teacher at the university level, students and many teachers at the pre-university level do not have the necessary training or experience to conduct animal-based research. Therefore, they should not engage in studies which would subject an animal to pain or discomfort, or interfere with its health and well-being. Comments on experimental studies involving animals at the pre-university level, as well as the names of qualified experts on animal care and use, may be obtained by writing to CCAC.

The Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals has been developed primarily for use at the post-secondary level; however, its principles apply equally well to pre-university work with experimental animals. Those planning to undertake such work should therefore become familiar with the contents of this guide and adhere to the guiding principles of the CCAC and the Youth Science Foundation (YSF).

The Youth Science Foundation (YSF), Suite 904, 151 Slater Street, Ottawa, Ontario K1P 5H3, is the umbrella organization responsible for all out-of-school science activities in Canada. Its objective is to develop scientific awareness among pre-university students as well as to encourage scientific literacy with the Science Fair Program, one of its principal activities.

YSF programs are directed primarily toward the English-speaking students in Canada's ten provinces and territories. The Conseil de developement du loisir scientifique (CDLS), 4545, rue Pierre du Coubertin, C.P. 1000, Succursale M, Montreal, Quebec, H1V 3R2, by mutual agreement with the YSF, is responsible for similar activities for Francophone students, primarily in the province of Quebec.

When the Canadian Council on Animal Care was established in 1968, the council, together with representatives from the Canadian Veterinary Medical Association in concert with the Youth Science Foundation, recognized the importance of well-conceived science fair projects involving live animals. Over the years, attempts were made to ensure development of proper scientific investigational attitudes as well as respect for living things. As these early efforts proved largely unsuccessful,

increasingly stringent requirements were made. Finally, in May 1975, the regulations now in effect, developed by the Animal Care Committee of the Youth Science Foundation and CCAC, were adopted.

The Canadian Council on Animal Care, following the acceptance of the regulations for animal experimentation in science fairs and their implementation, prepared guidelines governing the use of animals in the classroom at the pre-university level for use by departments of education and boards of education across Canada. Their aim was to ensure the existence of adequate safeguards for the proper care and use of animals in experimentation in the classroom.

Suggestions for experiments involving animals at the pre-university level are available from CCAC.

Additionally, collaboration has been undertaken with various high school educational authorities in the preparation of manuals and course outlines involving animals and teaching programs at the pre-university level.

Guiding Principles Governing the Use of Animals in the Classroom at the Pre-University Level

I. Purpose

These guiding principles have been adapted from the Canadian Council on Animal Care. The Nova Scotia Department of Education recommends their use to ensure adequate safeguards exist for the proper care and use of animals in the classroom.

These guidelines are not for use by students preparing projects for exhibit in science fairs. Students preparing projects for science fairs must adhere to the Youth Science Fair Regulations for Animal Experimentation, as prepared and distributed by the Youth Science Foundation, Suite 302, 151 Slater St., Ottawa, Ontario K1P 5H3.

II Philosophical Considerations

Biological experimentation involving animals in the classroom is essential for an understanding of living processes. Such studies should lead to a respect for all living things. All aspects of the study must be within the comprehension and capabilities of the students undertaking the study.

Lower orders of life are preferable subjects for experimentation at the pre-university level. Such lower orders as bacteria, fungi, protozoa, and insects can reveal much basic biological information; they should be used for experimentation, wherever and whenever possible.

III Care of Experimental Animals

The care of experimental animals in the school should embody the principles laid down in this guide.

Adherence to the following principles is necessary in order to provide optimal animal care:

a. Quarters specifically designed for housing of animals should be provided and should be appropriate for the needs of the species involved.

b. All experimental animals used in teaching programs must be properly cared for. Animal quarters should be made comfortable by provisions for sanitation, protection from the elements and sufficient space for normal behavioral and postural requirements of the species. The living quarters shall have surfaces that may be easily cleaned; good ventilation and lighting, well regulated temperatures; and cages of sufficient size to prevent overcrowding. Animals must be protected from direct sunlight or other environmental factors which may disturb their well-being.

c. Food should be palatable, and of sufficient quantity and balance to maintain a good standard of nutrition. Foods intended for animals must not be allowed to fall below the recommended maintenance level of nutrition. Clean drinking water must be available at all times. Containers for food and water should be of a design made specifically for that purpose.

d. Colonies and animal quarters shall be supervised by a teacher familiar with animal care. The students and other animal care staff must be trained and required to handle the animals gently and humanely.

e. All animals must be disposed of humanely. If any animal has to be destroyed, an approved humane method must be carried out by an adult experienced in the use of such procedures.

f. The use of animals must comply with existing local, provincial or federal legislation.

g. The procurement and use of wild animals and birds must comply with the Migratory Birds Convention Act of Canada, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (ratified by Order in Council July 3/75), as well as any existing legislation at the provincial level concerned with wild animals and exotic species.

IV. Experimental Studies

1. All experiments must be carried out under the supervision of a competent teacher. It is the responsibility of the teacher to ensure that students have the necessary comprehension for the study to be undertaken.

2. Students should not be allowed to take animals home for the purpose of carrying out experimental studies. All studies involving animals **must** take place in a suitable area in the school.

3. All students carrying out projects involving vertebrate animals must adhere to the following guidelines:

   A. No experimental procedures shall be attempted on a vertebrate animal that could subject it to pain or distinct discomfort, or interfere with its health.

   B. Students shall not perform surgery on vertebrate animals except for dissections of preserved specimens or specimens killed by a teacher (see Euthanasia)

   C. Experimental procedures shall not involve the use of:

      a. microorganisms that can cause diseases in humans or animals
      b. ionizing radiation
      c. cancer-producing agents
      d. drugs or chemicals at toxic levels
      e. alcohol in any form
      f. drugs that may produce pain
      g. drugs known to produce adverse reactions, side effects, or capable of producing birth deformities

   D. Experimental treatments should not include exercise until exhaustion, electric shock, or other distressing stimuli.

   E. Behavioral studies should use only reward (positive reinforcement) and not punishment in training programs.

   F. If egg embryos are subjected to experimental manipulations, the embryo must be destroyed humanely two days prior to hatching. If normal egg embryos are to be hatched, satisfactory humane provisions must be made for disposal of the young birds.

4. The use of anaesthetic agents by students is not recommended and, in the case of some anaesthetics, is not permitted by law.

5. Information on the care, housing and management of individual species, as well as suitable experiments for use at the pre-university level, may be obtained from the Canadian Council on Animal Care, 1000-151 Slater St., Suite 1105, Ottawa, Ontario. K1P 5H3.

Youth Science Foundation Regulations for Animal Experimentation in Science Fairs

1. Biological experimentation is essential for an understanding of life processes. Such studies should lead to a respect for all living things. Capable students, anxious to pursue a career in biological sciences, must receive the necessary encouragement and direction. All aspects of the project must be within the comprehensions and capabilities of the student undertaking the study.

2. Lower orders, such as bacteria, fungi, protozoa and insects can reveal much basic biological information. If experiments are to be conducted on living subjects for science fair projects then only lower orders of life may be used.

3. **Vertebrate animals are not to be used in experiments for projects or for science fairs, with the following exceptions:**

   A. Observations of normal living patterns of wild animals in their free habitats or in zoological parks, gardens or aquaria.

   B. Observations of normal living patterns of pets, fish or domestic animals.

4. No living vertebrate animal shall be displayed in exhibits in science fairs.

5. Cells such as red blood cells, other tissue cells, plasma or serum purchased or acquired from biological supply houses or research facilities, may be used in science fair projects. (NOTE: Guidelines from this document **PROHIBIT** the use of mammalian cells, tissues, plasma or serum.)

6. Observational studies on only chicken egg embryos may be used in science fair projects. If normal egg embryos are to be hatched, satisfactory humane provisions must be made for disposal of the chicks. If such arrangements cannot be made then the chicken embryos must be destroyed on the 19th day of incubation. No eggs capable of hatching may be exhibited in science fairs.

7. All experiments shall be carried out under the **supervision** of a competent science teacher. It shall be the **responsibility** of the qualified science teacher to ensure the student has the necessary comprehension for the study to be undertaken. Whenever possible, specifically qualified experts in the field shall be consulted.

Euthanasia

The term "euthanasia" is used to describe the process whereby an animal is killed using recognized, acceptable humane techniques. By derivation, it means "easy death" and thus carries the explicit implication of a quiet, painless death without fear or anxiety.

The most important criterion of acceptance of a euthanasia method as a humane process is that it have an initial depressive action on the central nervous system (CNS) to ensure immediate insensitivity to pain.

It is important to recognize that some methods of euthanasia, which cannot be made aesthetically pleasant, such as decapitation or stunning with excanguination (draining the blood), may nonetheless be humane in terms of the above criterion. This concept is important to keep in mind when deciding on the method of euthanasia to be used. The choice must be based on the sensibilities of the animal to be killed rather than the observer or operator, although the latter should not be disregarded.

APPENDIX II (b)

PROCEDURES FOR OBTAINING FRESH TISSUE SAMPLES FROM FISH, AMPHIBIANS AND REPTILES

When anesthesia or euthanasia is required to obtain tissue samples these procedures are to be performed by the teacher before the laboratory activity commences. Students should <u>not</u> perform or observe these procedures.

Handling, anesthesia and euthanasia methods specific to fish, amphibians and reptiles as prescribed by the CCAC, are as follows:

FISH

Handling Methods

1. Physical Restraint

Fish are covered with a thin, delicate cuticle, and damage to the skin represents a break in the osmotic barrier between fish and water. When handling fish, therefore, great care must be taken to avoid damaging the skin. The hands should always be wet, as should nets and all other materials coming into contact with the fish. In order to avoid abrasions to the skin of struggling fish, netting materials should not be too coarse or hard.

2. Chemical Restraint

Anesthesia, administered via the water, may be desirable if a lot of handling is necessary. Numerous anesthetics are available, including carbon dioxide and benzocaine; however, the one in most common use is tricaine methane sulphonate (MS-222 at a concentration of 1/12,000-1/25,000, depending on the depth of anesthesia and speed of induction required).

3. Bleeding

Blood samples may be obtained from anesthetized fish with no detrimental long-term effects. The two main sites for this are the caudal vein and the heart, with the former being preferable. The dorsal aorta, running along the roof of the mouth, is an alternative sampling site in salmonoids.

4. Euthanasia

Euthanasia may be accomplished by an overdose of anesthetic or by spinal severance just behind the head. For large fish, or where anesthetics are to be specifically avoided, a sharp blow on the top of the head, while firmly holding the fish out of water, is also considered a humane method.

AMPHIBIANS AND REPTILES

Frogs and toads should be picked up by the hind legs or, with experience, by the body; use of a cloth may be helpful. When it is necessary to pick up an axolotl, or other urodeles with external gills, particular care must be taken not to put pressure on them.

Because of their permeable skin, amphibians can be easily anesthetized by placing them in a solution of anesthetic. Tricane methane sulfonate (MS-222), as a 0.01% aqueous solution, will produce anesthesia within a few minutes, depending on the size of the amphibian.

Hypothermia is often recommended as a form of anesthesia in cold-blooded animals. Placing the animal in a refrigerator or ice water bath immobilizes it. A state of unconsciousness appears to be produced; however, it is not known whether this is actually so and whether analgesia is really induced. For these reasons, hypothermia should be used only for restraint for relatively non-painful procedures and not as an anesthetic for major surgery.

Blood Smears

A goldfish, frog, salamander, newt, lizard, turtle or tortoise could serve as a source of fresh blood for blood smear preparation. A fixed permanent human blood smear can be used for comparison.

The same procedure generally used for human blood smear preparation, fixing and staining should be followed, substituting the selected animal's blood. Wright's stain is the most commonly used and would work well. Other stains that might be tried are Leishman-Giesma, Lugol's, Jenner-Geisma, or Kleinberg-Nobel.

To obtain blood from a fish, amphibian or reptile, anesthesia by immersion in the appropriate solution concentration of MS-222 is necessary. The solution is prepared by dissolving the appropriate amount of MS-222 (TMS) crystals in water. When preparing the solution, **teachers or laboratory assistants should wear disposable gloves, and a mask over the nose and mouth area.** When immersing or removing the specimen from the anesthetic bath, gloves must be worn. Fresh solution is required for all procedures.

Safe disposal of used MS-222 solution requires that vermiculite be used to absorb the liquid, that it be bagged and labelled biohazardous, and then incinerated.

Anesthetizing amphibians and reptiles for blood sampling requires a 1:2000 solution of MS-222. Once the animal is anesthetized by immersion, blood may be obtained from frogs or toads by opening the mouth and extending the tongue forward, revealing the floor of the oral cavity. Clean out the mucus and use a lancet to pierce the lingual vein posterior to the hyoid cartilage. Blood can then be drawn up in capillary tubes containing a small amount of 10% citrate solution to prevent clotting. Blood can be distributed to the students' slides from the capillary tube. Bleeding will stop spontaneously when the tongue is placed back in the mouth. Put the animal back in fresh water immediately to recover.

Newts' or salamanders' blood can be drawn by piercing a toe with a lancet and collecting the flowing blood in 10% citrate solution capillary tubes as described above. The best spot for piercing a reptile's toe is at the junction of the terminal phalanx and the claw. Return the animal immediately to fresh water to recover. Bleeding of the animal will stop spontaneously.

A medium-sized goldfish can be immersed in a 1.0% solution of MS-222 which will kill it painlessly. A single goldfish can then serve to supply blood for the class as well as be a source of other tissue samples for microscopic examination. Blood may be obtained by removing the tail with a sharp knife and collecting blood from the dorsal aorta with capillary tubes containing small amounts of 10% of citrate solution (see diagram next page). Various other tissues can also be collected for examination. Epithelial cell scrapings from inside the cheek of the fish can replace human cheek cell scrapings. Cell squash preparations for gill tissue, liver, pancreas and other soft tissue areas can serve to show cellular differentiation. Thin cross-sections of muscle tissue and gut cross-sections can also be attempted. Methylene blue stain is effective for these temporary preparations. Gross gill structure, scale overlap and structure and tail capillary structure can also be observed.

Lancets must be properly disposed of in biohazard canisters. All used slides and surfaces must be decontaminated. Procedures for both are contained in the section Biohazardous Materials: Labelling and Proper Disposal Technique.

Make a swift cut completely through the fish at the position shown, slightly forward of the narrowest section between the trunk and tail.

To obtain blood easily from the caudal cut, tip the fish as shown and gently squeeze the anterior portion while touching the capillary tube to the dorsal aorta.

APPENDIX II (c)

SYNTHETIC URINE PROCEDURES

1. Synthetic urine used in experiments for detecting salts:

   Add 4-5 drops of yellow food coloring and 9.5g of NaCl (sodium chloride) to 1L H_2O (distilled).

2. Synthetic urine used in experiments for determining sugar concentration

   i) 0% $C_6H_{12}0_6$ (glucose) solution
   Add 4-5 drops of yellow food coloring to 1L H_20 (distilled)

   ii) 1% $C_6H_{12}0_6$ (glucose) solution
   Add 4-5 drops of yellow food coloring and 10 ml of $C_6H_{12}0_6$ (glucose) to 990 ml of H_20 (distilled)

   (iii) 4% $C_6H_{12}0_6$ (glucose) solution
   Add 4-5 drops of yellow food coloring and 40 ml of $C_6H_{12}0_6$ (glucose) to 960 ml of H_20 (distilled)

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